THE EFFECT OF INTRAPERITONEALLY APPLIED PURE MICROCYSTIN LR ON HAEMATOLOGICAL, BIOCHEMICAL AND MORPHOLOGICAL INDICES OF SILVER CARP (*Hypophthalmichthys molitrix* Val.)

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Abstract

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The aim of this study was to investigate the effect of intraperitoneally applied Microcystin LR to the health condition of silver carp *(Hypophthalmichthys molitrix Val.)* stockfish by means of assessment of haematological, biochemical and morphological indices.

Two-year-old stockfish of silver carp was used for experiments. In the first trial, pure Microcystin LR was applied to the fish intraperitoneally in dose of 250 $\mu g \cdot kg^{-1}$ body weight (bw). A pure Microcystin LR dose of 400 $\mu g \cdot kg^{-1}$ bw was used in the second experiment. After 48 hours, fish were sampled for blood by cardiopunction to determine the erythrocyte count (RBC), haematocrit (PCV), haemoglobin concentration (Hb), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH). The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and total protein concentration (TP) were determined from blood plasma.

The pathological image was similar for both experimental groups under study, with the most remarkable impairment of hepatopancreas (enlargement, yellow - brownish colour, injection of vessels or haemorrhages). Histological examination showed large dystrophic and necrobiotic alterations of hepatocytes, as well as of kidney tubuli.

Haematological examination showed a highly significant increase of plasmatic enzyme activity (ALT, AST, LDH), as well as a highly significant decrease in TP compared to the control group of fish. Moreover, a highly significant decrease of both haematocrit and haemoglobin concentration was recordered in the first experiment.

The results of our study indicate that the intraperitoneally applied Microcystin LR causes mainly a serious impairment of hepatopancreas in silver carp, shown as an increase in activity of plasma enzymes.

Cyanotoxins, plasma enzymes, red blood cells, histo-pathological changes

A mass development of cyanophytes creating a so-called "algal bloom" is one of the negative impacts of surface water eutrophication (Marvan and Maršálek 1996). Cyanophyta are defined as photosynthetic gramnegative eubacteria able to produce biologically active substances. Among them, the toxins of cyanophyta - *the cyanotoxins* (Maršálek and Turánek 1996) represent an important group. They are products of the secondary metabolism of cyanophyta and according to their toxicity they can be classified among toxins of higher plants and bacterial toxins. According to their biological activity, cyanotoxins are distinguished as neurotoxins, hepatotoxins, cytotoxins, genotoxins, immunotoxins and embryotoxins. Biological activity of the respective cyanotoxins is usually of a composed character and a population of a single species of cyanophyta can

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produce more types of toxins. Freshwater cyanophyta produce mostly microcystins and microviridins which are classified among hepatotoxins according to their effect. At present, there are 28 various microcystins known with the mostly widespread Microcystin LR - a product of *Microcystis aeruginosa* (Maršálek and Turánek 1996).

The effect of cyanotoxins was mostly described in homoiothermic vertebrates. The impairment of hepatocytes is the most expressive effect of hepatotoxins (Runnegar and Falconer 1986; Falconer and Yeung 1992) and it is reflected among others also in changes of activity of liver enzymes in blood serum. There is a significant increase of alanine-aminotransferase (ALT), glutamin-transpeptidase (GTP) and alkaline phosphatase (AP) (Maršálek and Turánek 1996).

The occurrence of cyanotoxins in recreation reservoirs and in drinking water represents an increasing danger for human. It is therefore necessary to find the most effective as well as the most sensitive way of eradication of the overpopulated blooms of cyanophyta. A few ways how to reduce a mass development of cyanophytes are described in the literature, one of them by reduction of their development using their natural consumers, the herbivorous fish. The silver carp (*Hypophthalmichthys molitrix* Val.) was most frequently tested for this purpose (Adámek 1981; Adámek and Spittler 1984; Adámek et al. 1990; Beveridge et al. 1993).

The effect of cyanotoxins on fish is mentioned marginally in the fish disease literature. A danger of oxygen depletion followed by suffocation of fish after mass decomposition of algal bloom is usually reported (Noga 1995; Roberts and Schlotfeldt 1986; Schäperclaus et al. 1979). Recently, few papers appeared on the studies of the effect of cyanotoxins on fish and aquatic animals (Bruno et al. 1989; Garcia 1989; Råbergh et al. 1991; Tencalla et al. 1994; Navrátil 1996; Navrátil et al. 1995, 1997). They summarize experimental results on the application of cyanophytes or cyanotoxins to trout or common carp (*Cyprinus carpio* L.). According to the anticipated possibility of utilizing silver carp for biological amelioration in eutrophicated reservoirs, we were using especially silver carp in our experiments with application of Microcystin LR or biomass of blue-green algae.

The silver carp (*Hypophtalmichthys molitrix* Val.) is a species of the family *Cyprinidae*. Adults grow up to 1 m length and 82 kg bw. It originates from rivers of Eastern Asia, from Amur River to rivers on the south of China. It has been introduced to the former Czechoslovakia in 1965. Adults are typically planktonophagous with phytoplankton totally dominating in their food. The fish is adapted morphologically for such feeding with gill rakers creating a very effective filtration apparatus and with its gut several times longer than the body length, thus enabling a complete utilization of the algal and cyanophyta biomass (Baruš et al. 1995).

Materials and Methods

The experimental fish (a two-years-old stock of silver carp) were kept in a Dubravius pond and transfered to $1 m^3$ fibre-glass tanks one week prior to experiments. The tanks were placed in an aquarium room, filled in advance with tap water and areated. Mean water temperature was 18 °C. Fish in Experiment 1 were transferred to 2 glass aquaria of 70 l volume each with the same water and aeration. Fish in Experiment 2 were left in two fibre-glass tanks.

Prior to the application of Microcystin LR, every fish was weighed and the dose was calculated individually according to fish weight.

In Experiment 1 (Sept. 30 – Oct. 2, 1996), 16 silver carp (8 experimental and 8 control fish) of 261 ± 40.0 g mean body weight (bw) were used. Experimental fish were treated intraperitoneally with pure Microcystin LR (Calbiochem, USA) dissolved in Aqua pro injectione in a dose of 250 $\mu g \cdot kg^{-1}$ bw. Control fish were treated with pure Aqua pro injectione. The treatment followed the approach of Čítek et al. (1997) as described for common carp: the injection site was on the left side of the fish on the point of intersection of two thought straight lines, the

first one from basis of the pectoral fin paralelly to the body axe and the second one from the middle of the ventral fin upright the first straight line. The needle was inserted cranially into the body cavity at a 30° angle. The experiment was terminated 48 hours after application.

Experiment 2 (June 4 - 6, 1997) was similar to the first one but 12 fish were used (6 experimental, 6 control ones) with mean bw of 430 ± 100.7 g. Experimental fish were treated intraperitoneally with pure Microcystin LR (Calbiochem, USA) similarly to Experiment 1 but in a dose of 400 μg · kg^{-1} bw.

All fish were clinically observed after the treatment and the experiment was terminated after 48 hours. All fish were consequently caught, fish blood was sampled by cardiac punction into single use syringes with 70 IU · ml^{-1} of Heparin Spofa. Blood samples were processed immediately. Fish were killed after sampling, pathologically and anatomically examined post mortem and tissue samples were taken for histological examination. Samples were fixed in a solution according to Bodian and processed after standard histological methods (dehydration in alcohol concentration series, embedded in paraffin, cut and dyed with Haematoxylin and Eosin).

Haematological examination included determination of erythrocyte count (RBC), haematocrit value (PCV), haemoglobin concentration (Hb) and computation of mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV). Methods used followed Svobodová et al. (1986). Biochemical examination of blood was focused on the determination of activity of plasmatic enzymes (alanine aminotransferase -ALT, aspartate aminotransferase - AST and lactate dehydrogenase -LDH) by means of kit tests (HUMAN) , as well as on the concentration of total protein in blood plasma (TP) photometrically by Bio-Lachema-Test (Lachema Diagnostika, CR).

For statistical evaluation, t-test was used (STATPlus programme, Veterinary Research Institute, Brno).

Results

Experiment 1

The experimental fish after intraperitoneal application of Microcystin LR did not show any clinical signs of intoxication within 48 h. Dissection showed that all experimental fish had a small amount of a pure haemorrhagic liquid in the body cavity. The most remarkable impairment was found on hepato-pancreas (enlargement, rounded margins, yellow - brownish colour and rarely haemorrhages). Histological examination of hepatopancreas showed dystrophic and necrobiotic alterations of hepatocytes with karyorrhexis and karyolysis. Dystrophic and necrobiotic alterations were found on kidney tubuli and cell detritus was registered in lumina of some tubuli. Tubuli of higher order were found more damaged.

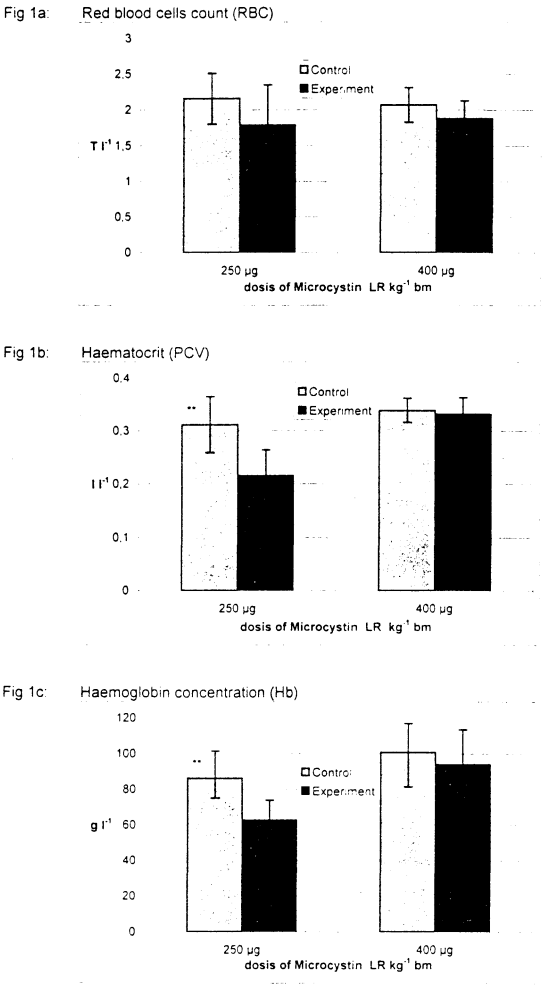


Fig. 1.
Mean values of haematological indices of silver carp, 48 h after i.p. administration of pure Microcystin LR (bars indicate SD)

Fig 1d: Mean corpuscular haemoglobin (MCH)

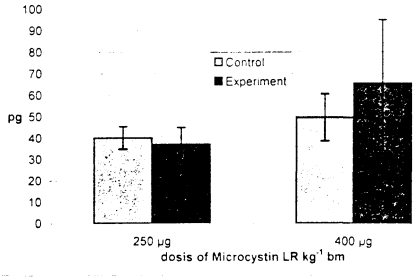


Fig 1e: Mean corpuscular haemoglobin concentration (MCHC)

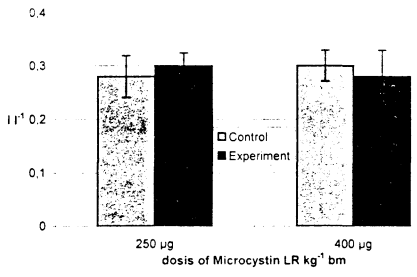
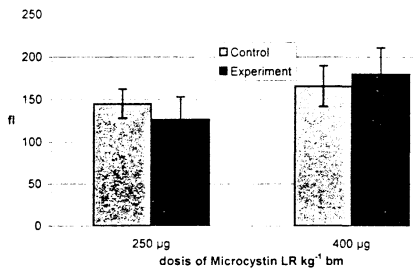


Fig 1f: Mean corpuscular volume (MCV)



Haematological examination showed a highly significant decrease of PCV ($p < 0.01$, Fig. 1b), Hb (Fig. 1c) and TP (Fig. 2d) of experimental fish compared to the control group. In contrary, the activities of all three plasmatic enzymes (ALT, AST, LDH) were found highly significantly increased ($p < 0.01$, Figs. 2a, 2b,2c). Differences in other indices were found insignificant: decrease of RBC, MCH, MCV and increase of MCHC (Figs. 1a,1d,1f and 1e).

Experiment 2

Within the 48 hours of study, the experimental fish showed signs of lassitude, swimming disorders and weakened escaping reflex. These disorders were most probably due to outbreak of bacterial infection (erythrodermatitis) prior to the start of the experiment, as there were similar signs found on the control group of fish. Macroscopical examination showed lesser/greater haemorrhages and suffusions on the skin and mainly on ventral part of the fish body, on fin bases and on the opercles. No changes were found on the intestines of the control fish while all fish of the experimental group showed enlargement of hepatopancreas of yellowish colour with haemorrhages and a small amount of a pure liquid was found in body cavity of three fish.

Haematological examination showed highly significant ($p < 0.01$) differences in biochemical indices of blood plasma, i. e. a decrease in TP (Fig. 2d) and increase of activity of plasmatic enzymes (ALT, AST, LDH; Figs. 2a, 2b, 2c). The RBC, Hk and Hb (Figs. 1a, 1b, 1c) were insignificantly lower, while MCH, MCHC and MCV were insignificantly higher (Figs. 1d, 1e, 1f).

Discussion

Results oltained in both experiments are comparable to those reported in the literature. Rodger et al. (1994) studied pathological alteration of brown trout (*Salmo trutta*) killed during a period of mass dying of algal bloom of *Anabaena flos-aquae* in Loch Leven, Scotland. They registered changes on gills (apposition of secondary lamellae and their oedem, exfoliation of epithelium from the basal membrane, epiphelial cell necrosis), liver (cell degeneration with pycnosis and karyorrhexis of hepatocytes, local inflammatory infiltration) and in kidney (extension of capillaries in glomeruli). However, this paper did not include haematological investigation. Råbergh et al. (1991) described pathological

alterations of common carp after intraperitoneal application of Microcystin LR. After application of sublethal doses of the toxin (130-300 $\mu g \cdot kg^{-1}$), large impairment of liver was found, with dilatation of intercellular space in the parenchyma by hydropic degeneration of hepatocytes. Dilatation of Bowman's capsules was found in kidney. After application of lethal doses of this toxin ($550\ \mu g \cdot kg^{-1}$), there was found a total destruction of the liver parenchyma, along with degeneration of kidney tubuli. Increased activities of ALT, AST and LDH were registered in blood plasma of the experimental fish. Navrátil (1996) and Navrátil et al. (1995) reported pathological alterations in stockfish of common carp after peroral application of pure Microcystin LR and/or the biomass of cyanophyta containing exclusively this cyanotoxin. These studies described expressive dystrophic alterations of hepatocytes with karyopycnosis, karyorrhexis and karyolysis, small focal necroses, haemorrhages and oedem. Dystrophic and necrotic alterations and desquamation of epithelium tubuli were found in kidney. The haematological investigation of carp proved an increased activity of both AST and LDH after application of pure Microcystin LR and of both ALT and AST after application of the biomass. Clinical, pathological and anatomical signs of intoxication of silver carp were less expressed compared to the data in the literature which might be given by application of lower doses of Microcystin LR. Or is the silver carp more resistant to the effect of Microcystin LR? This question can be answered only after a new series of experiments with a way of toxin application, an environment and time of the year as close to natural conditions as possible.

Fig 2a: The activity of alanine aminotransferase (ALT)

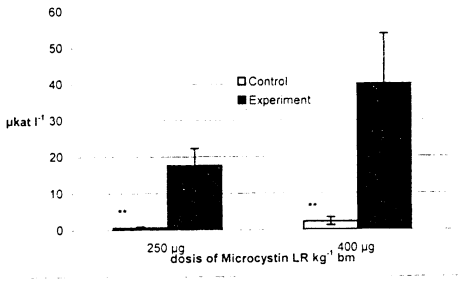


Fig 2b: The activity of aspartat aminotransferase (AST)

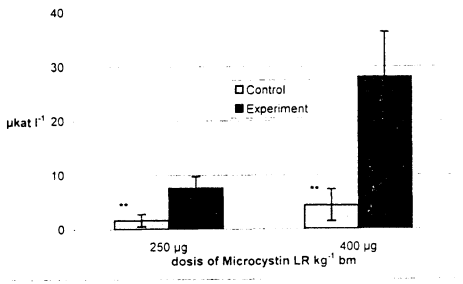
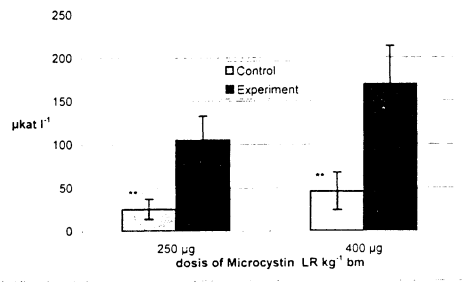
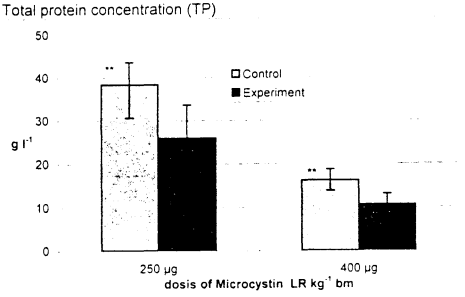


Fig 2c: The activity of lactat dehydrogenase (LDH)



** $p < 0.01$

Fig 2d: Total protein concentration (TP)



** $p < 0.01$

Fig. 2.
Mean values of biochemical indices of silver carp, 48 h after i.p. administration of pure Microcystin LR (bars indicate SD)

Vliv intraperitoneálně aplikovaného čistého Microcystinu LR na hematologické, biochemické a morfologické ukazatele tolstolobika bílého (*Hypophthalmichthys molitrix* Val.)

Cílem práce bylo zjistit vliv Microcystinu LR aplikovaného intraperitoneálně na zdravotní stav násady tolstolobika bílého (*Hypophthalmichthys molitrix* Val.) pomocí hodnocení hematologických, biochemických a morfologických ukazatelů.

K experimentům byla použita dvouletá násada tolstolobika bílého. V pokusu č.1 byl rybám intraperitoneálně aplikován čistý Microcystin LR v dávce 250 μg kg^{-1} živé hmotnosti ryb, v pokusu č. 2 byla použita dávka Microcystinu LR 400 μg kg^{-1} ž.hm. ryb. Po 48 hodinách byla rybám kardiopunkcí odebrána krev pro stanovení počtu erytrocytů (RBC), hematokritu (PCV), koncentrace hemoglobinu (Hb), střední barevné koncentrace (MCHC), objemu erytrocytu (MCV) a hemoglobinu erytrocytu (MCH). V krevní plazmě byla stanovována aktivita alanin-aminotransferázy (ALT), aspartát-aminotransferázy (AST), laktátdehydrogenázy (LDH) a koncentrace celkových bílkovin (TP). Po odběru krve byly ryby usmrceny a podrobeny patoanatomickému a patohistologickému vyšetření.

Patologický obraz obou pokusných skupin byl podobný - nejmarkantnější bylo poškození hepatopankreatu (zvětšení, žlutohnědá barva, nástřik cév nebo krváceniny). Histologické vyšetření odhalilo rozsáhlé dystroficko-nekrobiotické změny hepatocytů a v ledvinách dystroficko-nekrobiotické změny tubulů.

Hematologické vyšetření prokázalo v obou pokusech vysoce významné zvýšení aktivity plazmatických enzymů (ALT, AST, LDH) a vysoce významné snížení TP ve srovnání s kontrolními rybami. V pokusu č.1 bylo kromě toho zaznamenáno ještě vysoce významné snížení hematokritu a koncentrace hemoglobinu.

Získané výsledky svědčí o tom, že Microcystin LR po intraperitoneální aplikaci způsobuje u tolstolobiků bílých zejména vážné poškození hepatopankreatu, což se projevuje zvýšením aktivity plazmatických enzymů.

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